

SHORT COMMUNICATION

Evidence for epistasis: reply to Trouve *et al.*

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Salathé and Ebert (2003, *J. Evol. Biol.* **16**: 976–985) have shown that the mean logarithmic fitness of *Daphnia magna* clones declined faster than linearly with increasing inbreeding coefficient F . They interpreted this result as evidence for synergistic epistasis. Trouve *et al.* (2004, *J. Evol. Biol.*, doi: 10.1111/j.1420-9101.2004.00755.x) suggested that hybrid vigour could be an alternative explanation for this finding. We use a population genetic model to support the original claim that the marked deviation from linearity cannot be explained without epistasis. We further argue that the relevant reference population is the metapopulation and not the subpopulation. Taken together, we believe that synergistic epistasis between recessive deleterious alleles segregating in the *D. magna* metapopulation is the most likely explanation for the finding of Salathé and Ebert.

In a recent paper, Salathé & Ebert (2003) have shown that the mean logarithmic fitness of *Daphnia* clones originating from a particular crossing scheme (Fig. 1 in Salathé & Ebert, 2003) declined faster than linearly with increasing inbreeding coefficient F . They interpreted this result as evidence for synergistic epistasis between deleterious alleles at different loci; they asserted that in the absence of epistasis the relationship between F and mean log fitness should be linear. Trouve *et al.* (2004) notice that the three crosses whose fitness was assayed not only differed with respect to F , but also with respect to the expected contribution of the two parental lines to their genomes. They suggest that this should have led to hybrid vigour, which would have benefited certain types of crosses in our breeding design more than others. They claim that this hybrid vigour could be an alternative explanation for the nonlinearity of the relationship between mean log fitness and F . Hence, according to Trouve *et al.*, the conclusion about synergistic epistasis is not fully supported. Because hybrid vigour has been demonstrated in the *Daphnia magna* metapopulation from which Salathé & Ebert (2003) took their material (Ebert *et al.*, 2002), their idea needs careful consideration. The comments by Trouve *et al.* do not question our

second main conclusion, namely that there is no synergism between inbreeding and parasitism.

First we would like to point out that epistasis (like dominance) describes relationships between fitnesses (or phenotypes) of different genotypes. In contrast, hybrid vigour (like inbreeding depression) refers to a comparison of mean fitness between populations that differ in genotype frequencies. Both dominance and epistasis can contribute to both hybrid vigour and inbreeding depression (Lynch & Walsh, 1998). The magnitude of hybrid vigour and inbreeding depression depend on the choice of the reference population (see below for our choice of reference population). However, disregarding the reference population, whether the differences in fitness between lines assayed by Salathé *et al.* are described as inbreeding depression or hybrid vigour is of secondary importance for the conclusion about epistasis. The crucial question is whether the pattern in Fig. 4 of Salathé & Ebert (2003) can be explained without invoking epistasis.

Can the results be explained assuming no epistasis?

As pointed by Trouve *et al.*, the lines assayed by Salathé and Ebert differ in the genetic background: all genes of line G_{2s} descend from parental line P_s whereas in the backcrossed lines G_{2x} and G_{3x} a part of one haploid set of genes (respectively 50 and 25%) come from parental line P_x . A legitimate concern, not addressed in Salathé & Ebert

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(2003), is whether the linear relationship between F and fitness expected under no epistasis still holds in this case.

We will show that, although it does not hold in general, this relationship does hold for the three specific lines assayed by Salathé and Ebert. Specifically, we will show that without epistasis (i.e. with log fitness additive across loci), $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$ holds for an arbitrary set of genotypic fitness values, where w_i is expected log fitness of cross i as defined by pedigree in Fig. 1 in Salathé & Ebert (2003). Because the absence of epistasis means that effects on log fitness are additive across loci, it suffices to show that this relationship holds for each locus separately. Consider thus a single locus and, without loss of generality, denote the two alleles carried by line P_s A^1 and A^2 , and the allele passed on by line P_x to line G_{1x} A^3 . Of course, A^1 and A^2 will be identical if P_s is a homozygote, and A^3 may also be identical to one or both alleles carried by P_s . Let q_i^{kl} be the probability that line i carries genotype $A^k A^l$, and let $\mathbf{q}_i = [q_i^{11}, q_i^{12}, q_i^{22}, q_i^{13}, q_i^{23}]$ be the vector of these probabilities for all genotypes possible for a given descendant line. Note that, by symmetry and definition, $q_i^{11} = q_i^{22} = F_i/2$, where F_i is the inbreeding coefficient of line i relative to P_s . For the doubly selfed offspring G_{2s} it can be easily seen from the pedigree in Fig. 1 in Salathé & Ebert (2003) that

$$\mathbf{q}_{G_{2s}} = \left[\frac{3}{8}, \frac{1}{4}, \frac{3}{8}, 0, 0 \right]. \quad (1)$$

The backcross G_{2x} has a 50% probability of receiving A^3 , in which case the other allele is equally likely to be A^1 or A^2 . In the remaining 50% of cases, line G_{2x} will have received both alleles from parental line P_s ; they will be identical by descent in half of those cases, so

$$\mathbf{q}_{G_{2x}} = \left[\frac{1}{8}, \frac{1}{4}, \frac{1}{8}, \frac{1}{4}, \frac{1}{4} \right], \quad (2)$$

consistent with $F_{G_{2x}} = 0.25$. The second backcross G_{3x} has only a 25% probability of having received A^3 , and $F_{G_{3x}} = 0.5$, so

$$\mathbf{q}_{G_{3x}} = \left[\frac{1}{4}, \frac{1}{4}, \frac{1}{4}, \frac{1}{8}, \frac{1}{8} \right]. \quad (3)$$

Let \mathbf{w} be the vector of effects of the five genotypes on logarithmic fitness. The expected log fitness of line i is then $\bar{w}_i = \mathbf{q}_i \mathbf{w}^T$, and the difference in expected fitness between, e.g. G_{2x} and G_{3x} is $(\mathbf{q}_{G_{2x}} - \mathbf{q}_{G_{3x}}) \mathbf{w}^T$ etc. Note that

$$\mathbf{q}_{G_{2x}} - \mathbf{q}_{G_{3x}} = \mathbf{q}_{G_{3x}} - \mathbf{q}_{G_{2s}} = \left[-\frac{1}{8}, 0, -\frac{1}{8}, \frac{1}{8}, \frac{1}{8} \right], \quad (4)$$

which implies that $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$. Because at the same time $F_{G_{2x}} - F_{G_{3x}} = F_{G_{3x}} - F_{G_{2s}}$, a linear relationship between inbreeding coefficient and fitness is predicted for these three lines. This is valid for arbitrary fitness values, and for any pair of parental genotypes P_s and P_x , whether or not the three alleles are unique. Because in the absence of epistasis log fitness is additive across loci, the relationship $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$

holds for multiple loci. Furthermore, it also holds if the parental lines are genetically variable, irrespective of linkage disequilibria. Crosses between such variable population can be broken down into crosses between pairs of individual genotypes; as the eqn 4 holds for each such cross separately, it must hold for their sum.

It should, however, be stressed that the above is a special case. In general, in the absence of epistasis a linear relationship between log fitness and F is expected only if frequencies of all genotypes are linearly related to F . This is the case for the three focal lines (G_{2x} , G_{3x} and G_{2s}), but the other two lines (G_{1x} and G_{1s}) derived in the pedigree designed by Salathé & Ebert (2003, Fig. 1) do not fit this relationship. This is why the test for epistasis in Salathé & Ebert (2003) was only based on those three crosses, for which the relationship between log fitness and F was expected to be linear in the absence of epistasis. This was not clearly elucidated in that paper. In contrast to what Trouve *et al.* seem to suggest, whether the two parental lines originate from the same or different local populations is irrelevant for this conclusion.

Genetic independence of crosses

Trouve *et al.* also question Salathé and Ebert's design on the ground that the lines with different F were not genetically independent, which may have biased the statistical tests. There are two sources of this genetic nonindependence; we are not sure which Trouve *et al.* allude to, so we discuss them both. First, the tests were based on comparing fitness of lines (G_{2x} , G_{3x} and G_{2s}) from the same 'family', i.e. descendant from the same pair of parental lines P_s and P_x , replicated across six independent families. This is appropriate: the prediction of linear relationship between F and fitness applies to lines within a family, related to one another by the specific pedigree, and not to lines occupying the same pedigree position in different families. Deviations from this relationship are thus directly tested by comparing lines within families. The main effect of family (factor 'origin' in the analysis of variance, Table 1 in Salathé & Ebert, 2003) controls for the effect of genetic background (i.e. the identities of alleles A^1 , A^2 and A^3 in the above model). Secondly, the expectation of linear relationship is based on the probabilities of the three lines (G_{2x} , G_{3x} and G_{2s}) carrying specific genotypes (eqns 1–3), assuming that they are sampled independently. However, within each family each line was only represented by a single clonal genotype, and these genotypes were not sampled independently. For example, G_{3x} was the daughter of G_{2x} , so G_{3x} could not be $A^1 A^3$ if G_{2x} happened to be $A^2 A^2$. As a result, the sampling errors of fitness estimates of those three lines within a family are nonindependent. This indeed in principle violates an assumption of analysis of variance. However, fitness differences observed between those lines are likely to be due to a number of loci, at least some of which would

segregate independently (*D. magna* has 10 pairs of chromosomes). This should average out any potential bias due to nonindependent sampling. Still, to be on the safe side one can take a conservative approach, directly testing the null hypothesis $(\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}}) - (\bar{w}_{G_{3x}} - \bar{w}_{G_{2x}}) = 0$ with a *t*-test. For this test each family provides a single data point, so the nonindependence of data within a family is not an issue. This test rejects the null hypothesis both in the absence ($t = 2.78$, $P = 0.039$, d.f. = 5) and in the presence of parasites ($t = 6.98$, $P = 0.0009$, d.f. = 5), confirming the existence of a nonlinear relationship between fitness and *F*.

What is the relevant reference population?

Inbreeding coefficient and inbreeding depression are defined relative to a reference population, and the choice of the reference population should reflect the biology of the species (Keller & Waller, 2002). While Trouve *et al.* apparently consider the local subpopulation as the reference population, we believe that in our case the entire metapopulation constitutes the biological relevant reference population (Haag *et al.*, 2002). As shown above, the issue of the reference population is irrelevant to the conclusion that the pattern found by Salathé and Ebert implies epistasis. However, these epistatic interactions may have been between alleles originating from different local populations. So the ecological relevance of the pattern found by Salathé and Ebert would depend on how often alleles originating from different local populations meet in the same individual and thus have a chance to express their epistatic interactions. We believe that this happens frequently and therefore the metapopulation is the relevant reference population, a point on which Salathé and Ebert (2003) did not elaborate.

Salathé & Ebert (2003) used material from a highly dynamic metapopulation with average extinction probabilities of nearly 20% per local population per year (Pajunen & Pajunen, 2003). Populations go through extreme founder effects during colonization and suffer from very high drift loads (Ebert *et al.*, 2002). In the most extreme and, to our knowledge, the most frequent cases, one single clone founds a new population. To survive the following winter, members of this clone must sexually produce resting eggs (in this case by a process genetically equivalent to selfing), making the entire population highly inbred ($F = 0.5$) during that next year, even if it has expanded to a large size. Because the populations have, on average, a short time of survival, they do not have time to diverge more than they did through the founder effect: random genetic drift (other than through the founder effect) and the accumulation of mutations do not play a significant role. Thus, over evolutionary time the entire metapopulation shares a common gene pool,

even if at any time local populations may show a pattern of considerable differentiation. Hence, it is justified to regard the metapopulation as the reference population in assessing inbreeding depression. From this perspective, inter-subpopulation crosses (hybrids) are similar to crosses among randomly chosen individuals in a large outcrossing population. The difference between the fitness of inter-subpopulation crosses (hybrid vigour) and crosses within subpopulations is then equivalent to the difference between inbred and outbred lines within a large population.

Conclusion

To summarize, in the absence of epistasis the relationship in Fig. 4 in Salathé & Ebert (2003) should be linear, irrespective of hybrid vigour. In other words, the marked deviation from linearity cannot be explained without epistasis. The most plausible explanation is synergistic epistasis between recessive deleterious alleles segregating in the metapopulation of *D. magna*, from which the experimental clones originated. Although we believe that Trouve *et al.* (2004) highlight important points relevant to the understanding of epistasis, we do not agree with their suggestion that, for Salathé and Ebert's results, 'hybrid vigour is an explanation as likely as synergistic epistasis'.

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